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SECONDARY INFECTION IN PULMONARY TUBERCULOSIS. THE RECOVERY OF THE STREPTOCOCCUS AND PNEUMOCOCCUS FROM THE BLOOD.*†

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The degree of importance of secondary infections in pulmonary tuberculosis is a much disputed question. A certain group of observers consider the tubercle bacillus as responsible for practically all the pathological conditions in tuberculosis of the lung, while others believe that the secondary invaders, more often streptococci, pneumococci, and staphylococci, play a very large rôle in the production of the changes. Between these two extremes stand those who assign a certain import to both the primary and the secondary invaders: some holding the opinion that the major rôle is played by the tubercle bacillus, others that the tubercle bacillus is of lesser importance and that the major rôle is played by the secondary invaders.

There is a large amount of evidence advanced in support of each of these views. This evidence, however, is for the most part incomplete and contradictory. The complexity of the problem is such that many of the methods which have been employed are of little value in furnishing reliable data, and in reviewing the literature one is impressed with the great variety of conclusions drawn, even by those using the same methods of investigation.

Seven methods of attack have been variously employed in the study of secondary infection in pulmonary tuberculosis, namely: (1) the direct observation of clinical phenomena, (2) animal experimentation, (3) bacteriological and anatomical examination of the lung after death, (4) bacteriological study of the sputum, (5) study of the opsonic index, (6) study of the leukocytes, and (7) blood cultures.

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The study of the clinical phenomena has led to direct contradictions. Baumler,[1] for instance, believes the frequent occurrence of bronchopneumonia after hemorrhage to be due to secondary infection, while Sorgo[2] contends that the tubercle bacillus itself is capable of producing the bronchitis or pneumonia.

Czaplewski, Ziegler, Maragliano, Weichselbaum, and Strumpel (all cited by Cornet[3]) on the basis of clinical studies, believe that secondary organisms are intimately concerned in the pathology of chronic pulmonary phthisis and, on the same basis, Cornet and Petruschky[4] are convinced that the steep temperature curve frequently seen in chronic pulmonary tuberculosis is due to a mixed infection. On the other hand, Pick[5] points out that this type of fever has been observed in cases showing only tubercle bacilli in the sputum and no secondary organisms in the tissues after death.

Rapid emaciation, excessive weakness, cough, profuse expectoration, chills, and sweats are all attributed to the presence of secondary pyogenic organisms; on the other hand, individuals having many secondary organisms in the sputum and pronounced cavities may not show fever, sweats, or emaciation (Sorgo).

Likewise the evidence based upon animal experiment is in a large measure contradictory. Prudden,[6] for instance, found that the inoculation of tubercle bacilli into rabbits produced tuberculosis, but seldom caused cavities; the intratracheal injection of streptococci into tuberculous animals, however, caused marked cavity formation. Marmorek,[7] on the other hand, was able to produce cavities by injecting pure cultures of tubercle bacilli together with large quantities of their toxins.

The evidence contributed by bacteriological and anatomical examination of the lung after death is to a large degree unreliable—postmortem, agonal, and terminal invasions being complicating factors of marked frequency and extent. However, secondary organisms have been described in the walls of cavities where during life they kept pace with the tubercle bacillus, or even preceded it in the invasion of healthy tissue (Cornet). Kossel and Cornet have both found secondary organisms in tubercles in the liver and spleen widely separated from the original seat of infection.

Sputum examination has been employed extensively in the study of secondary infections in pulmonary tuberculosis, but this method again must be recognized as having sharp limitations. It is a well-known fact that all organisms present in mixed infections in tuberculosis may also be found in the healthy mouth, pharynx, and trachea. Whether these organisms may be present normally in the alveoli of the lung is an open question.[8] Little more weight can be given to the results obtained by examination of washed tuberculous sputum, although the results of Kitasato,[9] Petruschky,[10] Spengler,[11] Schabad,[12] and Cornet agree in designating the streptococcus as the secondary organism most constantly recovered in such examinations. Sorgo maintains that the amount of washing used by these observers was not sufficient, and according to his extremely stringent rules mixed infection is much more rare than the above observers are led to believe.

[1] *Deut. med. Wchnschr.*, 1893, 19, p. 1.
[2] *Ztschr. f. klin. Med.*, 1907, 51, p. 250.
[3] "Tuberculosis and Acute General Miliary Tuberculosis" in Nothnagel's *Practice of Medicine*, W. B. Saunders & Co., Philadelphia, 1904, p. 583.
[4] *Ztschr. f. Hyg.*, 1894, 17, p. 59.
[5] *Wien. klin. Rundschau*, 1905, 19, p. 253.
[6] *New York Med. Jour.*, 1894, 60, p. 1.
[7] *Compt. rend. Soc. de Biol.*, 1904, 66, p. 60.
[8] *Jour. Exp. Med.*, 1905, 7, p. 78.
[9] *Ztschr. f. Hyg. u. Infektionskr.*, 1891, 11, p. 441.
[10] *Op. cit.*
[11] *Ztschr. f. Hyg. u. Infektionskr.*, 1894, 18, p. 343.
[12] *Ztschr. f. klin. Med.*, 1897, 33, p. 476.

The opsonic index has been employed by Wirths[1] to determine the existence of a mixed infection in tuberculosis and also the identity of the particular secondary invader. In a series of clinical cases Wirths found the index constantly normal, i.e., between 0.8 and 1.2, in all cases to the *Diplococcus capsulatus*, *Micrococcus tetragenus*, *Micrococcus catarrhalis*, meningococcus, pneumobacillus, pseudodiphtheria bacillus, colon bacillus, *B. subtilis*, but abnormal to the influenza bacillus in two cases of 17 (12 per cent), to the pneumococcus in 18 cases of 24 (75 per cent), to the streptococcus in six cases of 19 (30 per cent). He found the index to the four last organisms normal in five cases of 25 (20 per cent). The actual value of the opsonic index is yet to be determined by a more extensive application; at all events, the results are not at present uniform in the hands of all workers.[2]

Leukocytosis in pulmonary tuberculosis has been employed as a criterion indicative of a mixed infection (Simon)[3] inasmuch as the tubercle bacillus alone, except in acute miliary tuberculosis, does not produce a leukocytosis. Pick, Warthin, v. Jaksch, and Galbraith found the number of leukocytes in uncomplicated tuberculosis normal or low. Strauer, Grawitz, Halbron, and Appelbaum found the leukocytes normal in incipient cases. Ullon and Craig[4] also made leukocyte counts on a considerable number of cases and found an average of 10,285 in cases of the first stage; 12,772 in the second stage; and 14,041 in the third stage. I have made leukocyte counts on 112 cases and found an average of 11,963 in 16 incipient cases, 14,783 in 84 advanced cases, and 15,820 in 12 far-advanced cases.

A certain grade of leukocytosis is not, of course, rare in chronic pulmonary tuberculosis and a distinct leukocytosis is perhaps indicative of a mixed infection; the limitation of the method in the study of secondary invaders in tuberculosis is, however, that a secondary invader may be present without producing an evident leukocytosis, and furthermore, that the leukocytosis when occurring gives no information concerning the character or location of the secondary invading organism.

Finally there remains to be considered the blood-culture method of investigation. It has long been recognized that the finding of secondary invaders in the blood stream in pulmonary tuberculosis would constitute most direct evidence as to the importance of the rôle played by such organisms, and blood cultures have therefore been repeatedly employed in attempts to show the existence of such a bacteremia. As in the case of the other methods heretofore cited, however, directly contradictory deductions have been made by those employing this method. But in this instance I believe that the confusion in results is due to variations in the technic used rather than to the method itself; and from a study

[1] *Beiträge z. klin. d. Tuberk.*, 12, p. 159.

[2] I have determined the opsonic index to the streptococcus, pneumococcus, and staphylococcus in 40 cases of pulmonary tuberculosis and have found the index between 0.8 and 1.2 in all cases but one, and in this case the index to the *Staphylococcus aureus* was 0.75. As in Wirths' work, heterologous strains were used; possibly the result would have been different had homologous strains been employed.

[3] *Clinical Diagnosis*, Lea Bros. & Co., 1907, Philadelphia and New York.

[4] *Trans. Nat. Ass. Study and Prevention Tuberculosis*, 1905, 1, p. 166.

of 130 cases of pulmonary tuberculosis of which this paper is a report, I am convinced that blood cultures give definite, positive proof that secondary invading organisms are present in the blood stream of a large percentage of individuals afflicted with pulmonary tuberculosis, and that the organisms so present play an extensive rôle in the production of the symptoms. Before proceeding to give details of the methods and results of my own work however, it may be well to analyze the previous attempts in this direction.

By way of introduction it may be stated that the investigation by means of blood cultures fall into two groups, namely, those of the earlier workers and those of relatively recent date. The early investigators obtained a high percentage of positive results. They secured the blood by pricking the ear or finger, and allowing a few drops to fall through the air into culture media. The chance for air and skin contamination was great, and the number of staphylococci (chiefly albus) isolated, indicates that the large percentage of their positive findings was due to such contaminations. On the other hand, the later investigators, using a more trustworthy technic, drawing the blood directly from a vein under attempted aseptic conditions, have shown a very low percentage of positive results—about 2.5 per cent.

Jakowski,[1] puncturing the finger and allowing the blood to drop into culture media, secured seven positive results in nine observations. Two of the positive cultures gave streptococcus; one, streptococcus and staphylococcus together; and four, staphylococcus.

Hewelke[2] found organisms in 14 out of 21 cases. Ten were staphylococcus; one case showed a diplococcus and one case a non-pathogenic organism, either a coccus or a short bacillus. In another series, using venous puncture, he obtained three positive results out of 13. These were all non-liquefying white cocci.

Petruschky[3] drew a definite amount of blood, and injected mice and inoculated culture media. He found streptococci in one case out of eight.

Sittman,[4] drawing one c.c. of blood from the vein at the elbow in four cases, obtained *Staphylococcus aureus* in three cases and *Staphylococcus albus* in one.

Schabad,[5] using the same method as Sittman, recovered *Staphylococcus albus* in one case out of three.

Kraus,[6] in 14 observations, obtained the *Staphylococcus albus* in one case.

Hirschlaff[7] found a staphylococcus in four of 35 cases.

Von Michaelis and Meyer[8] found bacteria in blood cultures from eight cases in

[1] *Centralbl. f. Bakt.*, 1893, 14, p. 762.
[2] *Ibid.*, 1896, 19, p. 563.
[3] *Deut. med. Wchnschr.*, 1895, 19, p. 317.
[4] *Deut. Arch. f. klin. Med.*, 1894, 53, p. 323.
[5] *Ztschr. f. klin. Med.*, 1897, 33, p. 476.
[6] *Ztschr. f. Heilkunde*, 17, p. 117.
[7] *Deut. med. Wchnschr.*, 1897, 13, p. 766.
[8] *Charitéannalen*, 1897, 22, p. 150.

10. Five of the organisms were staphylococcus, one was a diplobacillus, one a gram-positive diplococcus, and one a streptococcus. The blood was taken shortly before death—four, three, two, nine, and 14 days, for those recorded.

A. Fraenkel,[1] using Sittman's method, obtained negative results in all of 20 cases.

Schroeder and Naegelsbach,[2] putting one c.c. of blood into broth, obtained negative results in all of eight cases. The patients were far advanced and all died within one month after the taking of the blood.

Straus[3] found the blood sterile in 19 cases, although they were all in the last stages; most of them with marked remittent fevers.

Lasker,[4] plating two c.c. of blood in agar, obtained but one positive result in 68 cases. This case showed many streptococci.

Lemierre[5] found the blood cultures all negative from eight cases in the last stages.

Teissier,[6] investigating 53 cases, obtained nine positive results. He drew one c.c. of blood from an arm vein, and divided it among several tubes of gelatin and broth. Of the nine positive results, two were *Staphylococcus aureus*, three, streptococcus, and four were *Staphylococcus albus*.

Jockmann,[7] examining 40 cases, drew 20 c.c. of blood from the vein at the elbow, distributed this among six or seven tubes of agar at 45° C.; after shaking thoroughly, the contents were poured into Petri dishes and incubated at 37° C. All the results were negative. The patients showed various temperatures. Some were of a remittent type and cavities were present in almost all cases.

Panichi[8] found pneumococci in four cases of some 35 examined. He drew scarcely one c.c. of blood. These were all advanced cases; but one lived for seven months after the blood was taken. Panichi concludes that bacteremia may occur before the agonal period and be intercurrent. Pneumococci were found in one case that did not give a history of previous pneumonia, and he concludes that it was an organism coming from a cavity.

Benohr,[9] investigating 187 cases of tuberculosis, making 241 examinations, obtained four positive results. He drew 20 c.c. of blood and plated it in glycerin agar.

F. Reiche,[10] making 365 examinations on 288 cases of high fever in terminal stages, took 15 to 20 c.c. of blood and obtained 1.65 per cent positive results.

The workers following Sittman, for the most part, drew blood directly from a vein and their results are therefore to be held the more reliable in that the possibilities of contamination were greatly reduced. However, many of the positive results of these workers undoubtedly included contaminations, namely staphylococci from the skin. If, however, the staphylococcus findings are eliminated from the results of Tessier, von Michaelis and Meyer, Hirschlaff,

[1] *Berl. klin. Wchnschr.*, 1898, 35, p. 345.
[2] *Münch. med. Wchnschr.*, 1899, 46, p. 1339.
[3] *Semaine méd.*, 1894, 14, p. 253.
[4] *Deut. Aertzte-Zeitung*, 1901, 1, p. 27.
[5] *Bull. et mém. Soc. Med. de l'Hôp. de Paris*, 1903, 20, p. 1437.
[6] *Jour. de physiol. gén.*, 1901, 3, p. 223.
[7] *Deut. Arch. f. klin. Med.*, 1905, 83, p. 558.
[8] *Berl. klin. Wchnschr.*, 1908, 41, p. 1840.
[9] *Mitt. a. d. hamb. Staats-Krankenanst.*, 1908, 13, p. 323.
[10] *Med. Klinik*, 1909, 5, p. 1962.

Kraus, Lasker, Schabad, Sittman, Panichi, Jockmann, Reiche, and Benohr and others, I believe their positive results are reliable; for, as discussed later, streptococci and pneumococci are not organisms furnished by skin contamination. Excluding the staphylococci, however, the results of these later workers show a very low percentage of positive cultures and it has been concluded that if secondary infection is of importance at all, its influence is due to soluble toxins escaping from a localized infection in the lung, and not to a bacteremia.

My own experiments, as stated, lead me to quite the opposite conclusion, namely, that the invasion of the blood stream by pyogenic organisms is frequent in pulmonary tuberculosis.

In carrying on my work I have given emphasis to three main points: (1) the drawing of a large quantity of blood under the most favorable aseptic conditions, (2) the systematic inoculation of the most favorable media with considerable amounts of this blood, and (3) the rigid exclusion as positive cultural results of all organisms having a possible origin in skin or air contamination.

To cover the first point the following technic was employed in obtaining the blood for cultural purposes:

From 5 to 20 c.c. of blood, usually the latter amount, was drawn from an arm vein by means of a glass aspirating bulb of about 25 c.c. capacity. A cotton plug was placed within one of the ends and a piece of heavy rubber tubing about six inches long attached to this end. A second, shorter piece of rubber tubing was attached to the other end. An antitoxin needle (No. 18 caliber) was inserted into the distal end of the shorter piece of tubing and the tubing tightened about the hilt of the needle by binding with a rubber band. The needle was protected by slipping a test tube over the end.

Shortly before the bleeding, the whole aspirator, including the tubings and needle with a test tube over it, was wrapped in a towel and sterilized in the autoclave at 120° C. for at least 15 minutes.

In drawing the blood the upper arm was encircled tightly with a rubber bandage, the cubital region was scrubbed vigorously with alcohol,[1] anesthetized quickly with ethyl chloride, and the venous puncture made. When the blood commenced to flow into the aspirator, the compression of the upper arm was relieved, and the blood aspirated into the bulb by suction through the heavy rubber tube attached to the cotton-plugged end of the aspirator.

[1] Various methods of skin sterilization were tried, but none was found that was more efficient than the scrubbing with alcohol. In a number of cases the skin of the cubital region was scrubbed thoroughly with green soap, and rinsed with five per cent carbolic acid and 1/1000 mercury bichloride. In another group of cases the cubital region of the patient's arm was scrubbed with green soap and painted with tincture of iodin.

The percentage of cultures showing *Staphylococcus albus* after these methods of skin preparation was as great as in the cases in which simple cleansing of the skin with alcohol was used.

As soon as the required amount of blood had been withdrawn, the needle was removed from the vein, a wire sterilized in the flame was inserted to occlude the lumen of the needle, and the whole needle then heated to redness. This, with the resulting coagulation of the blood, effected a sealing of the needle.

In making the transfer from the aspirator to the culture media, the short rubber connection and the needle were detached and the end of the aspirator heated thoroughly in the flame. The corks of the culture tubes and flasks were flamed, the corks removed by an assistant, the necks of the tubes and flasks heated thoroughly in the the flame and the desired amount of blood was then transferred quickly from the aspirator to culture flask. The end of the aspirator was flamed again before more blood was transferred to a second culture flask. In spite of a rigorous enforcement of this technic as a precaution against contamination in drawing the blood and in transferring it to the culture media, contamination by staphylococci occurred in 10 per cent of the cases. The staphylococcus undoubtedly represented a skin contamination and in a control series of 21 blood cultures made on normal individuals, a similar contamination occurred in two instances.

In the attempt to grow organisms from the blood obtained in the manner above given, the following cultural methods were employed. Five c.c. of agar was sterilized in eight-ounce flat flasks, and immediately before using, the agar was melted and cooled to 40° C. From one to two c.c. of blood was introduced into each of one, two or three of these flasks containing melted agar, and after the blood and agar were thoroughly mixed by gently agitating, the flasks were laid on the side and the agar allowed to harden, giving a large layer of blood agar, about $\frac{1}{8}$ in. in depth. The flasks were next put in the incubator at 37° C.

In a majority of the cases cultures were also made in broth,[1] usually five c.c. of blood being put into 50 c.c. of broth. No particular attention was paid to an exact dilution other than to avoid a concentration greater than one to 10. As a usual routine two or three such inoculations of broth were made. In a few cases flasks of litmus milk were used, but the broth was found to be much more satisfactory.

The residuum of the blood not employed in the above inoculations was incubated in the sealed aspirator or in a sterile test tube.

After 24 hours at 37° C., the blood-agar flasks were examined with a low-power lens with especial reference to the presence or absence of deep colonies showing hemolytic zones. In the case of a positive finding, typical colonies were transferred to blood-agar slants. The broth was examined after 48 hours. Microscopic examination was made of smears stained with simple gentian violet and by Gram's method. Blood-agar plates were inoculated with one c.c. of the 48-hour broth regardless of whether the microscopic findings were positive or negative. In a considerable number of instances, milk tubes also were inoculated from the broth. The full blood, incubated for 24 hours in the aspirator, was examined microscopically for organisms, and whether the finding was positive or negative, transfers were made at once to blood-agar slants. After incubation of the residuum of full blood for an additional 24 hours (total 48) this was plated in blood agar.

The blood-agar transfers obtained as above indicated from the original blood agar and broth and from the incubated full blood, were, in turn, incubated at 37° C. for 24 hours. The resulting growths were, in all instances, streptococci, pneumococci, or staphylococci.

[1] Mallory and Wright ,*Technic.*, 3d ed., Saunders & Co., 1904, pp. 71, 73, 74.

1. Cultures showing a clear, dewlike growth, confined to the needle track; or scattered, clear, fine, pin-point colonies were examined microscopically for a characteristic arrangement of the organisms in chains or in pairs. Transfers of individual colonies were made to plain-agar, potato, gelatin, milk, broth, dextrose-agar, and serum-inulin-agar, or serum-inulin-water and blood-agar and the cultural study of the fully isolated organism was continued in each instance until its identity was established. Daily descriptions of the growths were recorded for six consecutive days.

2. The 24-hour blood-agar cultures, which in contrast to those described above, presented an abundant surface growth, were examined microscopically for staphylococci, and were transferred at once to the usual series of culture media. An organism forming a white or yellow growth on plain agar, distinct growth on potato, liquefaction of gelatin, a considerable cloudiness in broth, or a white or yellow surface-growth on dextrose agar, was in all instances assumed to be a staphylococcus, was identified as such and was discarded as presumably representing a skin contamination.

As above stated the only organisms obtained in the original cultures of the entire series of examinations were staphylococci, streptococci, and pneumococci; and inasmuch as the staphylococci were eliminated, the results recorded as representing actually positive blood cultures have to do only with streptococci and pneumococci. On this basis alone, however, positive results were obtained in 46 per cent of the cases examined (60 in 130). The streptococcus was found in 36 cases and the pneumococcus in 24 cases.

It is not possible within the scope of this paper to give in detail the bacteriological results for the entire number of positive cases. The following six instances, however, serve as examples of the application of the methods outlined above and the results obtained.

"P."—Incipient pulmonary tuberculosis. Temperature normal. Ambulatory. Disease passive. Hemorrhagic history. Family history indicates marked predisposition to tuberculosis. Marked infiltration of right lung; moderate impairment of upper portion of left lung.

Cubital region of arm scrubbed with green soap and painted with iodin. Immediately before making venous puncture, operator's hands, covered with gloves, were dipped in 95 per cent carbolic acid and rinsed in mercury bichloride, 1/1000. Ten c.c. of blood was drawn from median basilic vein and two c.c. transferred to each of four flasks containing five c.c. of melted agar cooled to 40° C. Agar and blood were thoroughly mixed, and plates made by laying the flasks on the side. When agar had hardened the flasks were put in the incubator at 37° C. After 18 hours, numerous greenish colonies with hemolytic zones were seen on the plates.[1] Several such colonies were transferred to blood-agar slants. Blood-agar slants after 24 hours showed discrete, greenish, hemolytic colonies, shown by microscopic examination to be composed

[1] After 72 hours, plates were somewhat overrun with surface growth of white colonies. Smear of this latter growth showed large cocci in bunches. Identified as *Staphylococcus albus*.

of gram-positive cocci arranged in long chains. Several of these discrete colonies were transferred from the agar slants to a series of media with the following results:

SUBCULTURES AFTER 24 HOURS.

Blood agar: Few very small, dewlike, clear colonies. No increase in growth after 96 hours.

Plain agar: Same as blood agar, only much less extensive, practically invisible. No increase in three days, slightly increased in four days.

Potato: No growth in six days.

Gelatin: No growth in six days (room temperature).

Litmus milk: No apparent change.

Broth: No apparent growth.

Dextrose agar: No surface growth; questionable growth along stab.

Serum-inulin-agar: No change.

Diagnosis: Streptococcus.[1]

"D."—Advanced pulmonary tuberculosis. Condition passive. Ambulatory. Hemorrhagic history. Moderate infiltration of both lungs.

Cubital region of arm scrubbed with alcohol and 20 c.c. of blood drawn from median basilic vein. Transferred two c.c. immediately to an agar flask containing five c.c. melted agar cooled to 40° C. and transferred five c.c. to each of three flasks containing 50 c.c. of broth. The remaining blood was sealed in the aspirator and incubated without diluting.

SUBCULTURES AFTER 24 HOURS.

Blood agar: Showed many greenish colonies with hemolytic zones. Several colonies were transferred to blood-agar slants. Smears from 18-hour blood-agar slants showed diplococci in short chains and cocci in bunches. Growth on various media showed streptococci with *Staphylococcus-albus* contamination. A colony from the original blood-agar decolorized milk in 24 hours, and the smear showed many capsulated diplococci; some in short chains. Pure culture.

Broth: After 72 hours transfers were made to blood-agar slants. These subcultures, after 24 hours, showed fine, compact, opaque growths. Smear showed diplococci in short chains and groups. The cultural characteristics, staining reactions, and morphological arrangement of the organism isolated from flask No. 2 identified it as a streptococcus.

Full blood: Microscopic examination after 48 hours of the three c.c. of undiluted blood incubated in the sealed aspirator showed diplococci in short chains. Transfers were made to blood-agar slants, which after 24 hours showed slight, compact, greyish-looking growth. Smears from the same showed gram-positive diplococci in long chains,

[1] The differentiation of the streptococci from the pneumococci—in so far as that is possible—was based upon the following criteria: (1) Appearance of colonies on blood-agar slants; (2) Appearance of colonies on blood-agar plates, green colonies with imperfect hemolysis (fuzzy border) were considered pneumococci, while clear or opaque colonies showing zones of sharply demarcated hemolysis were considered streptococci if showing other suggestive characteristics; (3) Chain formation on blood agar and in broth; (4) Reaction on serum-inulin-agar (Ruediger) or in serum-inulin-water (Hiss). Growth in gelatin capsule formation, growth on plain agar, and morphology, were taken into account in making a differentiation, although they were not considered of distinct diagnostic value. It need hardly be added that applying all possible criteria, the validity of an absolute decision, as to which one of these two groups a given organism belonged to, was questionable.

which were plated in blood agar, with resulting colonies showing distinct clear zones of hemolysis. Transfers were made to a series of media with the following results:

Blood agar: 24 hours—fine, dewlike, compact growth along needle track; 48 hours—growth slightly increased.

Potato: No growth.

Litmus milk: 24 hours—slightly acid(?); 72 hours—acidified and coagulated.

Gelatin: No growth in three days.

Broth: No growth. Smears after three days negative.

Dextrose agar: No surface growth. Growth along stab(?).

Serum-inulin-water: No acid production.

Diagnosis: Streptococcus.

"C."—Advanced pulmonary tuberculosis. Disease active. Ambulatory. Marked infiltration of right lung.

Twenty c.c. blood was drawn and two c.c. was transferred to each of two flasks containing five c.c melted agar cooled to 40° C., five c.c. were put in each of two flasks containing 50 c.c. of plain broth, and remainder of the blood was put in a sterile test tube.

Blood agar: Both were sterile after 48 hours.

Broth: Microscopic examination of flask No. 1 showed gram-positive diplococci in short chains. Transfers were made to other culture media after 48 hours with results as follows:

Blood agar: Very scant, scarcely visible growth of fine, dewlike colonies after 24 hours; slightly increased after 72 hours. Smear showed gram-positive diplococci. Pure culture.

Plain agar: No growth.

Potato: No growth.

Gelatin: No growth.

Milk: 24 hours—acid; smears showed gram-positive diplococci. Pure culture.

Broth: Clear, but with pellicle. Smear showed moderately long chains of gram-positive cocci (12–24 cocci in a chain). Bacillus seen. Second tube was inoculated; slightly cloudy in three days. Gram-positive diplococci in chains were seen. Pure culture.

Dextrose agar: No growth.

Serum-inulin-water (Hiss): Acid in four days.

Diagnosis: Pneumococcus.

"G."—Far-advanced pulmonary tuberculosis. Condition very active. Large cavity. Several bad hemorrhages. Bed-ridden. Marked infiltration of both lungs, more pronounced in left.

25 c.c. of blood was drawn with usual technic and three c.c. of blood was transferred at the bedside to each of four flasks containing five c.c. of melted agar cooled to 40° C. Five c.c. of blood was transferred to each of two flasks of 50 c.c. broth. The remaining blood was sealed in aspirator and incubated undiluted.

Blood agar: After 24 hours the flasks showed many small, greenish colonies imbedded in the blood agar and also a surface contamination. Transfers were made from several of the deep colonies to blood-agar slants but the subcultures showed contamination with staphylococci. Discarded for pure cultures obtained from the full blood as below.

Broth: After four days the broth showed typical lanceolate diplococci, gram positive. No subcultures were made.

Full blood: Smears showed diplococci in long chains at the end of 24 hours. Subcultures were made on a series of media with the following results:

Blood agar: Fine, clear, dewlike, moist, elevated colonies; growth confined to needle track. Growth increased after 48 hours. Smear showed gram-positive diplococci in long chains.

Plain agar: 24 hours—very scant, almost invisible growth; 48 hours—growth somewhat increased; six days—no further change. Smear, after 24 hours, showed long chains of diplococci.

Potato: No growth.

Gelatin: No growth.

Litmus milk: 48 hours—acid. Not coagulated in six days.

Broth: 48 hours—visible growth. Slight cloudiness with granular sediment in six days.

Dextrose agar: Scant surface growth(?); scant growth along stab. No gas.

Serum-inulin-agar: Acid production in three days.

Diagnosis: Either pneumococcus or streptococcus—doubtful as to which.

"K."—Far-advanced pulmonary tuberculosis. Very active. Bed-ridden. Marked infiltration of right lung. Cavity.

Five c.c. blood was drawn and about two c.c. of blood run into a flask containing five c.c. of melted agar cooled to 40° C. After the blood was mixed with agar it was plated in a thin layer by laying flask on its side. The remainder of the full blood was sealed in the aspirator and incubated.

Blood agar: Negative after 48 hours.

Full blood: A transfer was made from the full blood to a blood-agar slant, after 24 hours. Twenty-four hour blood-agar slant showed scattered, small, greyish, opaque colonies. Smear showed cocci in chains. Transferred to other media.

SUBCULTURES.

Blood agar: Scattered, small, greyish, opaque, elevated, moist colonies after 24 hours. Very scant growth. Not increased in five days.

Plain agar: Very scant growth of clear, dewlike colonies. Growth slightly increased in 72 hours.

Potato: No growth in six days.

Gelatin: No growth in six days.

Litmus milk: Acid in 24 hours, fine coagulum in four days.

Broth: Fine, granular sediment; fluid clear. Smear after three days showed gram-positive cocci in long chains.

Dextrose agar: Scant, clear, surface growth. Slight growth along stab(?).

Litmus-inulin-agar: No acid.

Diagnosis: Streptococcus.

Blood culture made again one week later and the streptococci were again recovered.

"V."—Moderately advanced pulmonary tuberculosis. Active. Ambulatory. No hemorrhage. Marked infiltration of right lung. No cavities.

About 10 c.c. of blood was drawn and three c.c. was transferred immediately to each of three flasks containing five c.c. of melted agar cooled to 40° C. After mixing blood thoroughly with agar, plates were made by laying flasks on the side. After 24 hours at 37° C., several green colonies surrounded by clear zones appeared in two of the flasks, the third flask remaining sterile. Some of these colonies were transferred

to blood-agar slants, and from these, after 24 hours, subcultures were made on various media with results as follows:

Blood agar: 24 hours. Scattered, flat, green colonies, which clung tenaciously to the culture medium.[1] Colonies had a truncated-cone appearance. A smear showed capsulated diplococci. Growth increased after 48 hours. No further change in six days.

Plain agar: Very small, clear, scattered colonies in 24 hours. No change in six days.

Potato: No growth.

Gelatin: No growth.

Litmus milk: Acid production at 24 hours. Coagulation in three days. Smear showed gram-positive diplococci, lanceolate in shape.

Broth: No growth.

Dextrose agar: Good growth along needle track. No surface growth.

Diagnosis: Pneumococcus.

The details of the above six cases are representative of the findings in all of the 60 cases from which pneumococci or streptococci were isolated.

Twenty-three of the 60 strains isolated were tested as to their pathogenicity for mice, and 16 were found lethal.

Fifteen strains of the 60 isolated were employed also in conjunction with the homologous sera to determine whether or not the presence of the organism in the blood stream had modified the opsonizing value of the serum. In the case of 10 of the 15 sera (66 per cent) the opsonic index for the homologous organism was abnormal, namely, below 0.8 or above 1.2.

Number	Organism	Opsonic Index
1	Streptococcus	0.85
2	Pneumococcus	1.3
3	Pneumococcus	0.7
4	Streptococcus	0.95
5	Pneumococcus	0.5
6	Pneumococcus	0.7
7	Streptococcus	0.8
8	Pneumococcus	0.75
9	Pneumococcus	0.5
10	Pneumococcus	1.3
11	Streptococcus	0.8
12	Streptococcus	0.75
13	Streptococcus	0.7
14	Streptococcus	0.8
15	Streptococcus	0.7

As mentioned previously, a total of 130 cases, including instances of all stages of pulmonary tuberculosis, from passive incipient involvement to far-advanced active processes with cavity forma-

[1] In several cultures, organisms were isolated which displayed this characteristic.

tion, were examined. Streptococci and pneumococci were recovered from the blood in 60. The relation of the bacteriological findings to the various stages of the disease are given in the following summary:

Classification	Number of Cases Examined	Number of Positive Blood Cultures	Percentage of Positive Blood Cultures
Incipient.....	12	2	16
Advanced.....	99	45	45
Far advanced.....	19	13	68
Total.....	130	60	46

An analysis of this summary shows that positive blood cultures were obtained in a relatively small number of cases classed as "incipient," whereas the percentage was almost three times as great among the "advanced" cases. From the "far-advanced" cases the percentage of positive results was almost twice that among the "advanced" cases, and four times that of the "incipient" cases; 58 of the 60 positive cultures were obtained from "advanced" and "far-advanced" cases.

The general relation of the bacteriological findings to the grade of fever displayed by the host is expressed in the following statement:

Grade of Fever	Number of Cases Examined	Number of Positive Blood Cultures	Percentage of Positive Blood Cultures
Afternoon temperature below 100° F....	65	22	34
Afternoon temperature above 100° F....	65	38	58

From this it is seen that approximately two-thirds of the positive cultures were obtained from individuals showing a very distinct afternoon fever.

Could it be shown that the streptococci and pneumococci obtained in the above results have a possible source in air or skin contamination, as is actually the case with the staphylococci for instance, the above findings would be of little or no import in the discussion of secondary invaders. Hektoen[1] has rightly pointed out, however, that such contaminations with organisms other than the staphylococci, if occurring at all, are of such extremely rare occurrence in cultures with blood obtained by venous puncture as

[1] *Jour. Am. M. Ass.*, 1903, 40, p. 683.

to be a negligible factor. Rarely, indeed, is it possible to obtain streptococci by direct inoculations from the skin, even in the absence of surface sterilization. Thus Weaver,[1] attempting to cultivate this organism from the skin in a group of 18 scarlet-fever cases, was successful in but one case, and Dreyer, in a similar group of 30 cases, failed to cultivate the streptococcus in a single instance.

To control this point under fully parallel conditions, however, blood cultures were made from 21 normal individuals, employing exactly the same technic as that used in the case of the 130 tuberculous patients, even to the amount of blood withdrawn—10 to 20 c.c. The results obtained were in accord with the above statements, namely: Neither streptococci nor pneumococci were encountered in a single instance; whereas *Staphylococcus pyogenes albus* was present in two cases.[2]

In view of these facts, the conclusion seems warranted that the pneumococci and streptococci isolated by the blood-culture method used in the present instance had their origin not in an extraneous source, but actually in the circulating blood stream.

The high percentage of positive results in the series of cases here reported contrasts sharply, to be sure, with the results of previous workers who have employed the blood-culture method in studying secondary infections in pulmonary tuberculosis. The explanation of this difference I do not seek to establish other than to point out that in the present series a large number of cases was examined, a large amount of blood was used, and finally the cultural conditions most favorable to the growth of the organisms in question were rigorously maintained.

The isolation of the pneumococcus or the streptococcus from the blood stream of more than one-third of the cases examined leads me to the conclusion that not only are true secondary invading organisms of frequent occurrence in pulmonary tuberculosis, but further, that in many instances these organisms, entering the blood stream, constitute a complication of extensive pathological significance.

[1] Cited by Hektoen.

[2] The skin of the forearm of six normal individuals was entered with sterile needles which were then plated in blood agar. In two instances, there was a growth of the *Staphylococcus pyogenes albus*, the other four plates remaining sterile.